zic5 gene. We have generated in situ probes that are specific for the *zic1*, *zic2*, *zic3*, and *zic4* genes in chick. While there is considerable overlap in the expression patterns of these genes, there are also distinct differences. *zic2* and *zic3* are strongly expressed in the entire dorsal neural tube, including the brain, trunk, and tail tip. *zic1* expression is strong in the dorsal brain, but weaker in the dorsal neural tube of the trunk. *zic4* appears to be expressed exclusively in the head region. During somite development, *zic1* is expressed dorsomedially in epithelial somites, while *zic2* expression begins well after somites have given rise to distinct dermomyotome and sclerotome regions. *zic1–3* are expressed strongly in the dorsomedial parts of more mature somites, particularly in the posterior portions. *zic2* is the only family member expressed in the periotic mesoderm in the hindbrain. *zic2–4* are expressed in the developing eye and all *zic* genes are expressed in the optic stalk. Among the *zic* genes, only *zic2* is expressed in the limb buds. We are currently analyzing *zic3* and *zic4* expression in more detail.

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Program/Abstract # 172
A microarray screen for direct targets of the Zic1 transcription factor

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The transcription factor zic1 plays important roles in a variety of developmental processes. In vertebrates, these include patterning the early neural plate, development of the neural crest, somite development, and formation of the cerebellum. In addition, zic1 promotes cell proliferation. To increase our understanding of the molecular mechanisms that underlie these processes, we have conducted a DNA microarray screen with Affymetrix gene chips to identify downstream target genes of zic1. The screen was performed using *Xenopus* ectodermal explants. By using an inducible zic1 construct (zic1GR) and the protein synthesis inhibitor cycloheximide, the screen was designed to discover direct targets of the Zic1 transcription factor. One new gene that was identified in this screen is the putative protease Xfeb that contributes to hindbrain patterning. We give a general overview of the genes identified in this screen and will discuss our findings from the screen with respect to genes involved in neural crest development and in anterior–posterior patterning.

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Program/Abstract # 173
Requirement of *Goosecoid* in early *Xenopus* development: A loss-of-function study

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Goosecoid (*Gsc*), a homeobox gene, was the first gene found to be specifically expressed in Spemann's organizer. In the present study, we show that knock-down of *Gsc* in *Xenopus* by Morpholino oligomers (MO) leads to loss of head structures and expansion of ventral tissues. Unlike in the mouse, our results demonstrate a requirement of *Gsc* for head formation and axis patterning in early *Xenopus* development. In search of downstream mediator genes, we found that the effects of *Gsc* completely depend on the BMP antagonist Chordin. Ectopic expression of mouse Gsc mRNA lead to induction of a secondary axis. Co-injection of Chordin MO prevented this inductive capability of Gsc mRNA in 97% of the embryos. Evidence for regulation of Chordin by Gsc is further provided by quantitative PCR and in situ hybridization, showing that Gsc MO decreases *Chordin* expression in gastrulating embryos. Moreover, loss-of-function experiments in animal cap explants revealed mutual repression of Gsc and the ventral homeobox transcription factors Vent1 and Vent2. MO-mediated knock-down of Vent1/2 caused strong dorsalization of the embryos, which could be suppressed by Gsc MO. Our results suggest that Gsc and Vent1/2 have opposing activities during gastrulation and act as regulators of each other in mesoderm patterning.

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Program/Abstract # 174
TFIIH trafficking and its nuclear assembly during early Drosophila embryo development

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We present the analysis of the dynamic of the transcription factor IIH (TFIIH) during early *Drosophila* embryo development. TFIIH consists of ten sub-units: cyclin-dependent kinase 7 (Cdk7), cyclin H and MAT1 (CAK), two helicases Xpb/Hay and Xpd, and p34, p44, p52, p42, p62 and p8 (core). We found that the TFIIH core is initially located in the cytoplasm of syncytial blastoderm embryos, after the mitotic division 10 and until the cellular blastoderm the core moves from the cytoplasm to the nucleus. The CAK complex has a different behaviour in early embryonic stages. The CAK is mostly cytoplasmic during cellularization and even during gastrulation. However, both components are positioned at promoters of genes that are activated at the onset of transcription. Later in development, the CAK complex becomes mostly nuclear and co-localizes in most chromosomal regions with the TFIIH core, but not in all sites, suggesting that the CAK complex could have a role in transcription of some loci without interaction with the TFIIH core. We also demonstrate that the CAK and the core coexist in the early embryo cytoplasm, however they do not interact until they are in the nucleus. Our data suggests that the complete assemble of the ten sub-units TFIIH occurs during the activation of zygotic transcription. In addition, we demonstrate that the

nuclear entry of the core subunits XPB and XPD depends on their mutual interaction in the cytoplasm in the early embryo and this interaction is required for the onset of transcription.

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Program/Abstract # 175

A 17 nucleotide conserved sequence in VegT anuran orthologues

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VegT is the most important determinant of the endo-mesoderm in early frog development. For this reason we cloned orthologues from *Pipa pipa*, *Colostethus machalilla*, *Gastrotheca riobambae*, and the urodelean *Ambystoma mexicanum* and compared them with the orthologues from *Xenopus laevis*, *Eleutherodactylus coqui* and *Rana pipiens*. We have identified functionally conserved sequences within this transcription factor by examining the amphibian VegT orthologues against zebrafish (*Tbx16* or spadetail) and chicken orthologues (*Ch-Tbx6L*). The comparison revealed a totally conserved 17-nucleotide sequence near the 3′ end of the anuran open reading frame; the urodele sequence has one nucleotide difference. To test whether this conserved sequence is functionally significant for the transcriptional activity of VegT, we designed two truncated messages that included the 17 nucleotides conserved sequence and that were also missing 27 or 49 amino acids of the C-terminal end. Transcriptional activity was tested by plasmid in vitro RNA synthesis, animal cap assays, and the rescue of depleted embryos. These truncated messages have a significantly reduced transcriptional activity, suggesting that the 17 nts are partially required for this function. It may be that the conserved sequence interacts with the cytoskeleton or is a target for regulation by microRNAs. The specific function of the highly conserved 17 nts, however, remains unknown.

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Program/Abstract # 176

Comparison of Lim1 protein expression in embryos of four different frogs

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We developed a polyclonal antibody against *Xenopus* Lim1, and stained embryos in whole mount, to determine whether the dorsal lip of the blastopore is molecularly equivalent in the marsupial frog *Gastrotheca riobambae*, the dendrobatid frog *Colostethus machalilla*, and the leiuperid frogs *Engystomops randi* and *Engystomops coloradorum*. Developmental processes are retarded in *G. riobambae* and in *C. machalilla*; whereas in *E. randi* and *E. coloradorum* the rate of development is similar with that of *Xenopus laevis*. In embryos of *X. laevis*, this antibody recognized the Lim1 protein as a LIM homeodomain protein expressed in the dorsal blastopore lip of the gastrula, and at later stages, in the notochord, in specific neural cells, and in the pronephros. This protein expression pattern is similar with mRNA localization of the *Xlim1* gene. Surprisingly during gastrulation we encountered differences in the time and pattern of Lim1 expression. In the embryos of all species, however, Lim1 marks the presumptive dorsal side, as in *X. laevis*. At later stages of development, the expression of Lim1 was highly conserved in the notochord, neural cells, and pronephros. The different timing of Lim1 expression suggests that these frogs have evolved their own gastrulation processes by modifying gene expression patterns. This is the first molecular comparison of gene expression in the dorsal blastopore lip of frog embryos.

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Program/Abstract # 177

Ldb1, in conjunction with transcriptional regulators of the LIM-homeobox gene family, orchestrates limb patterning and outgrowth during mouse embryonic development

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We ablated the function of the LIM-homeobox transcription factors Lhx2, Lhx9 and Lmx1b by Cre-mediated deletion of their obligatory cofactor Ldb1 in posterior mesoderm-derived cells of the developing limbs. While both forelimb and hindlimb development was affected, more severe phenotypes affecting all three limb axes were observed in the developing hindlimbs. There was normal expression of Fgf10 in the limb mesoderm and Fgf8 in the apical ectodermal ridge (AER) shortly after limb initiation. The AER degenerated at E11.5. In situ hybridization revealed normal expression of Fgf10 in the limb mesoderm and Fgf8 in the AER shortly after limb initiation. This was followed by quick loss of AER markers and a strong reduction in the expression of genes that are specific for patterning and growth along the proximo-distal, dorso-ventral and antero-posterior axes. Rescue experiments with Fgf8 and Shh beads failed to restore normal mesenchymal Fgf10, Shh and Gremlin expression, respectively. By contrast, Fgf10 beads were able to restore normal Fgf8 expression in the AER. These data demonstrate that Ldb1 is required for gene expression induced by Fgf8 and Shh in the limb mesoderm. We conclude that Ldb1, in conjunction withLhx2, Lhx9 and Lmx1b, acts as a central node to